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Adsorptive stripping voltammetric assay of folic acid in human serum*

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Abstract: Folic acid was voltammetrically measured after preconcentration by adsorption at the static mercury drop electrode. Phase selective AC voltammetry provided the most sensitive stripping signal. After previous analyte extraction, using liquid–solid extraction with a C-18 reversed phase cartridge, the method was suitable for folic acid measurement in human serum. The detection limit was 5.9×10^{-9} M with an overall precision of 9.9% (R.S.D.; $n = 7$) at the concentration level of 1.0×10^{-7} M, with a mean recovery of 57%.

Keywords: *Folic acid assay; adsorptive stripping voltammetry; human serum.*

Introduction

Folic acid, *N*[4[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic acid, has long been recognized as a part of the vitamin B complex and is administered in the prophylaxis and treatment of megaloblastic anaemia and other diseases.

Voltammetric measurements of analytes previously preconcentrated by adsorption on the electrode surface provides a suitable technique for quantitation of a large variety of organic molecules at ultratrace levels [1]. The extremely high sensitivity and good precision attained by this technique, together with its instrumental simplicity, make it an attractive analytical tool for therapeutic monitoring purposes. The development of this kind of application needs to overcome problems arising from the presence of large excesses of naturally occurring surfactants in biological matrices such as serum. Competitive coverage of the electrode surface by matrix surfactants can hinder the adsorptive preconcentration process and even suppress the stripping signal. Recently, these problems have received important attention, and some methods have been proposed for trace drug monitoring in serum and urine samples [2, 3].

Experimental conditions affecting the adsorptive stripping behaviour of folic acid on mercury electrodes have been examined in a previous paper [4]. The present contribution describes an adsorptive stripping voltammetric assay allowing the selective measurement of the folic acid concentration in serum samples in the presence of reduced folates.

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Experimental

Apparatus

Voltammetric measurements were carried out using a Metrohm E506 polarograph coupled with a Metrohm 663 static mercury drop electrode (SMDE). Drop areas of 0.47 mm^2 were used. Potentials are referred to a saturated calomel electrode (SCE).

Reagents

Acetate buffers (0.1 M pH = 5.0) were used as background electrolyte. Folic acid, folinic acid, and 5-methyltetrahydrofolic acid were purchased from Sigma and used without further purification. Stock solutions (1.0×10^{-3} M) in NaOH 0.01 M were prepared daily and protected from the light. Analytical grade reagents and Milli-Q (Millipore) purified water were used.

Examined biological materials consisted of pools of human serum from at least 15 healthy individuals. Samples consisted of aliquots of 1 ml of pooled serum spiked with appropriate amounts of folic acid to achieve the desired final concentration.

Procedures

Sample purification was accomplished by liquid–solid extraction according to the following procedure:

The sample was diluted to 10 ml with pH 5.0 acetate buffer and mixed gently. The resulting solution was passed through a reversed-phase C-18 cartridge (Sep-Pak, Waters) previously activated by washing with pure methanol (10 ml) and water (20 ml). The effluent was discarded, the cartridge washed with 20 ml of water and the retained materials eluted with 2 ml of pure methanol, and the eluate collected in a 10 ml test tube. The solvent was evaporated to dryness at 60°C under an inert gas stream. The dry extract was dissolved in 10 ml of background electrolyte by shaking the tube mechanically for 2 min at room temperature, transferred to the electrochemical cell and, after degassing by purge with oxygen-free argon for 10 min, the adsorptive stripping voltammetric procedure was applied using the following instrumental set-up: preconcentration for 70 s in open circuit and quiescent solution; measurement of adsorbed analyte by phase-selective alternating current voltammetry at a frequency of 75 Hz, with 20 mV amplitude of superimposed alternating potential. The potential between −0.350 and −0.750 V was scanned at a rate of 10 mV s^{-1}, recording the stripping curve in the sampled current mode (sampling interval:0.4 s). The stripping current of the folic acid was measured at −0.650 V. Quantitation was achieved either by referring to a calibration graph or by the standard addition method.

Results and Discussion

A cyclic voltammogram of folic acid in adsorption-controlled conditions, concentration less than 1.0×10^{-5} M, shows three peaks corresponding to the three reduction processes involved (Fig. 1). The first reversible one, whose adsorptive stripping behaviour has been characterized elsewhere [4], was analytically exploited for the determination of folic acid in human serum. Differential pulse (DP), alternating current (AC), and linear sweep (LS) potential scan modes were tested for the stripping of adsorbed folic acid. The results obtained from a 1.0×10^{-7} M solution after a preconcentration time of 60 s indicated that the AC signal is 25 times larger than that

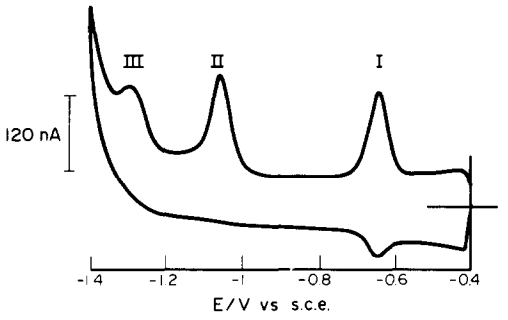

Figure 1
Cyclic voltammogram of folic acid from a 4.0×10^{-6} M aqueous solution; pH = 5.0. Scan rate: 100 mV s^{-1}.

obtained with a DP scan with 50 mV of superimposed voltage amplitude. This ratio is increased to 1000 when comparison is done with respect to LS voltammetry. This fact is in good agreement with the Laviron predictions for substances undergoing reversible reduction processes, in which both oxidized and reduced forms are strongly adsorbed on the electrode surface [5]. Given its extraordinary sensitivity, AC voltammetry has been used to monitor the folic acid in human serum.

Preliminary preconcentration experiments in serum extracts showed two stripping peaks corresponding to unknown matrix constituents, at −0.460 and −0.630 V, the latter being very close to the potential of the folic acid. The shape and the stripping current of the first one were strongly dependent on the preconcentration potential. When the preconcentration was carried out under electrolysis with an applied potential varying from 0 to −0.4 V, a broad stripping peak was developed, masking the second one. When the preconcentration was done in open circuit, the first peak was thinner and better resolution was achieved with respect to the second one, and consequently with respect to the folic acid.

Mass transfer conditions during the preconcentration step markedly influenced both the sensitivity of the resulting stripping signal, and the resolution of the first unknown peak appearing at −0.460 V with respect to that of the folic acid. In fact, when preconcentration was carried out under convective mass transfer conditions, the resolution worsened and the accurate measurement of the folic acid stripping current required the lowering of the amplitude of the superimposed alternating current to 5 mV. By contrast, if the preconcentration was accomplished in a quiescent solution, the resolution was substantially improved, the use of a 20 mV superimposed voltage being possible in order to record the folic acid signal. Furthermore, the detection limit of folic acid in human serum extracts was lower when the preconcentration was carried out in quiescent solutions, due to the smaller interference signal. The overall assay precision was also implemented by preconcentrating this way.

Under these conditions, plots of the stripping current versus the first power and the half power of the preconcentration time, for stirred and unstirred solutions, respectively, provided straight lines closely proportional to the folic acid concentration assayed, as shown in Fig. 2.

Figure 3 shows the AC voltammograms of serum blank and serum sample extracts obtained following the above proposed procedure. The stripping signal corresponding to the folic acid present in the sample responds linearly to external standard additions. All these facts come to prove the feasibility of carrying out the preconcentration of the analyte in quiescent solutions in such a complex matrix as a serum extract.

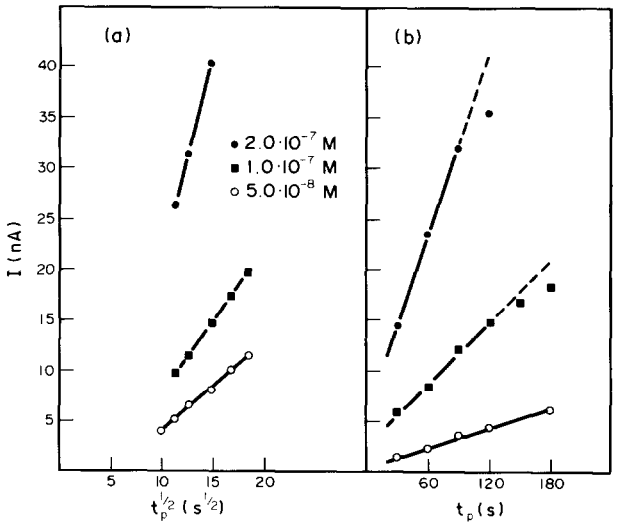

Figure 2
Effect of the preconcentration time (t_p) on the AC stripping current for extracts of serum samples containing different concentrations of folic acid. Preconcentration was carried out in (a) quiescent solution, and (b) in a stirred solution (50 Hz). pH = 5.0, scan rate = 10 mV s^{-1}.

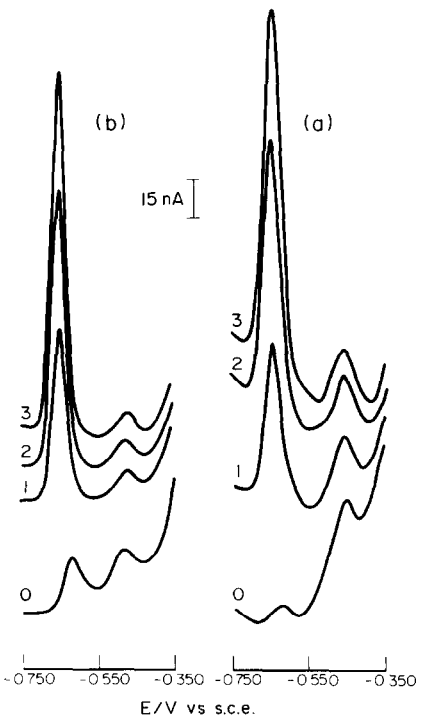

Figure 3
AC voltammograms of (0) a serum blank, and (1) a serum sample (44.14 ppb) extracts. (2) and (3) were obtained after standard additions of 0.88 ppb and 1.77 ppb respectively. Other conditions as in Fig. 2.

Investigations carried out in order to study the possible interference of reduced folates, mainly the 5-methyltetrahydrofolic acid (the naturally occurring folate in serum [6]), and 5-formyltetrahydrofolic acid, yielded a negative result when these substances were present in a 50-fold excess.

The proposed analytical procedure applied to 9 serum samples spiked with increasing amounts of folic acid covering a concentration range of two orders of magnitude from 2.0×10^{-8} to 2.0×10^{-6} M, leads to a wide linear dynamic range in the response of the stripping peak current with the concentration. The data reflecting these results, together with the relevant analytical data are listed in Table 1.

To the best of the authors' knowledge, this is the first report in which alternating current adsorptive stripping voltammetry, AC-AdSV, has been applied to the determination of a molecule of important biological significance in human serum.

Table 1
Relevent analytical data

Limit of detection (LOD)*	5.88×10^{-9}M
Limit of quantitation (LOQ)†	1.22×10^{-8}M
Linear range	2.0×10^{-8}–2.0×10^{-6}M
Calibration plot	(I/nA) = 0.31 + $8.69 \cdot 10^{7}$(C/M); $n = 9$; $r = 0.999$
Measure precision	1.02% (R.S.D., $n = 5$, at a serum concentration level of 1.0×10^{-7}M)
Overall assay precision	9.9% (R.S.D., $n = 7$, at a serum concentration level of 1.0×10^{-7}M)
Mean recovery	57%

*Obtained according to the criterion $S_t = S_b + 3.\sigma_b$ (ref. 7).
†Obtained according to the criterion $S_t = S_b + 10.\sigma_b$ (ref. 7).

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